IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

MacLEAN et al		Atty. Ref:	**620-73**
Serial No.	**08/776,350**	Group:	**1642**
Filed:	**April 18, 1997**	Examiner:	**Ungar**

For: **TREATMENT OF CANCER USING HSV MUTANT**

* * * * * * * * *

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

RULE 132 DECLARATION

I, S. Moira Brown, BSc. Ph.D. FRCPath, FRSE, hereby declare:

1) I am Professor of Neurovirology at University of Glasgow, University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital NHS Trust, Glasgow, G51 4TF.

2) I am an inventor of at least one claim of patent application no. 08/776,350. I have reviewed the pending claims of the above-identified application as well as the Remarks of the Amendment filed April 5, 2002 and the Neuropathology reports attached thereto. To the extent the Neuropathology reports or results presented in the Remarks of the Amendment of April 5, 2002, and specifically the

sentence spanning pages 3-4 of the Amendment filed April 5, 2002, may be taken as a suggestion that the patients of these studies were treated, *in vivo*, with the indicated virus, is in error. The following provides a more complete description of the study protocol and results indicated in the Neuropathology reports and graphs attached to the Amendment filed April 5, 2002.

3) I have investigated the action of ICP34.5 null HSV, e.g. HSV 1716, in metastatic brain tumours. I have confirmed or had confirmed at my direction that metastatic brain tumours from diverse origins support HSV 1716 infection *in vitro* and that the mode of tumour cell death is by virus replication and cell lysis. I believe that cerebral metastatic tumours of any origin should be treatable by HSV 1716. The work carried out to confirm these conclusions is outlined below.

4) Immediately after surgical excision, brain tumour specimens were collected from the operating theatre in accordance with current hospital R&D ethical guidelines, in ice-cold biopsy collection medium which consisted of Ham's F12 medium supplemented with 20mM HEPES buffer, 200 U/ml penicillin, 200ug/ml streptomycin, 100ug/ml gentamicin and 2.5ug/ml Fungizone (all from Invitrogen-Life Technologies, Paisley, UK). Approximately 1ml of tissue was usually harvested.

5) The tumour tissue was dispersed, normally within an hour of removal, following the method of Farr-Jones *et al* (J Neurooncol. 1999 May; 43(1):1-10 with slight modifications. Beginning with three gentle washes in ice-cold HBSS to

remove excess blood, followed by paring of any blood clots the tumour tissue was sliced using crossed scalpels to yield approximately 1mm^3 fragments. After another wash the fragments were resuspended in 30ml HBSS and digested with constant agitation for 30 min each at 37 °C and 4 °C with a cocktail of enzymes: collagenase (0.25mg/ml; Invitrogen-Life Technologies, Paisley, UK), pronase (0.5mg/ml), and DNase (0.4mg/ml; both from Sigma-Aldrich, Poole, UK). Any undigested material was sieved out with a 100µm pore nylon mesh and the suspension was layered on to 2x12ml Ficoll-paque (Amersham Pharmacia, Little Chalfont, UK) density gradient cushions and centrifuged at 400g for 30 min at RT. Tumour cells settled as a band at the interface and were siphoned off, whilst the erythrocytes sedimented at the bottom of the tube and were easily eliminated. Tumour cells were washed once with HBSS and the pellet resuspended in HBSS for viability checks.

6) The viability of dispersed cells was determined by the Trypan blue exclusion method (Freshney *et al.*, Cell. 1994 Sep 23; 78(6): 1039-49). Viability scores were regularly high, falling between 87.5% and 98.7%, except for the diathermy specimens.

7) Included for comparative purposes were 5 human cancer cell lines, a mouse embryo fibroblast cell line (3T6) and baby hamster kidney cells (BHK-21 clone13). The MCF-7 (breast adenocarcinoma), SCOV-3 (ovarian adenocarcinoma), LNCaP (prostatic carcinoma), HT29 (colonic adenocarcinoma), and C8161 (metastatic melanoma) cell lines were propagated

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in media prescribed by the American Tissue type Culture or the European Collection of Cell Cultures.

8) Tumour biopsy cultures were seeded at $2x10^6/cm^2$ in DMEM: F12 (1:1; Invitrogen-Life Technologies) supplemented with 10% FCS, 100µM sodium pyruvate, 0.05mM non-essential amino acids, 2mM L-glutamine (all from Invitrogen-Life Technologies), 100U/ml penicillin, 100µg/ml streptomycin and 2.5µg/ml Fungizone and incubated overnight at 37 °C, 5%CO_2, 99% humidity. Any unattached cells were removed and fresh medium was added. Permanent (cancer) cell lines were seeded at the same density in the prescribed medium.

9) BHK, 3T6 and the tumour cells under investigation, seeded at $2x10^5$ cells per 35mm dish, were infected the following day at a multiplicity of infection of 0.1 pfu/cell with the HSV-1 wild type strain 17 and with the ICP34.5 null mutant HSV1716. After adsorption of virus for 1h, the plates were washed once with PBS and overlaid with 2ml of growth medium. At 0, 6, 24, 48 and 72h post-infection the cells were scraped into the growth medium, sonicated and stored at –70°C. The samples were titrated on BHK cells to determine the amount of infectious virus present, as described elsewhere (Brown *et al.*, J Gen. Virol. 1973; 18: 329-346; Harland & Brown, 1997. In: Methods in Molecular Medicine Book Series: Herpes Simplex Virus Protocols, (eds) S.M. Brown & A.R. MacLean, Humana Press, New York). The BHK and 3T6 cells constituted the fully permissive and non-permissive controls in the assay.

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10) Metastatic brain tumours including 3 melanomas, 3 adenocarcinomas and 4 carcinoma in patients ranging in age from 20-71 years (mean: 53 years) were cultured. For comparative purposes, 4 cases of glioblastoma multiforme and a number of established tumour cell lines were included. The mouse embryo fibroblast cell line 3T6, which is selectively non-permissive for HSV1716 replication was also included (Brown *et al.*, J Gen. Virol.1994; 75: 2367-2377) (See Table 1 attached).

11) Metastatic brain tumour cultures were mostly of an undifferentiated flat epithelioid morphology unlike the glioblastoma (GBM) cultures which had the distinctive appearance of glial-like cells. Tumour growth usually began as islands which expanded to produce confluent cultures. Even at passage III (4-5 weeks), cultures showed little fibroblast overgrowth.

12) Assays were usually carried out on cultures at passage II. In the majority of primary tumour cultures, HSV strain 17 and HSV1716 replicated with similar kinetics giving final infectious virus yields of the same order. In a minority, HSV1716 replication was markedly impaired. Attached are growth curves and pathology reports for patients suffering from cerebral metastatic tumours. Patients' personal details have been removed and are now identified by case numbers. The case numbers are also used in Table 1. The attached shows growth curves of HSV17^{+} and HSV1716 in a fully-permissive culture (case 2) and in one which was selectively less permissive for HSV1716 (case 6). The replication kinetics are compared with those in BHK cells (fully permissive for

HSV1716) and growth arrested 3T6 cells (non permissive for HSV1716). The mean 72h yield from 10^6 BHK cells (calculated from 11 separate experiments) of HSV17$^+$ was 1.14 x 10^9pfu, whilst that of HSV1716 was 7.53 x 10^8pfu. The average yield (over 7 separate experiments) of 17$^+$ in 3T6 cells was 1.46 x 10^8 pfu/10^6 cells compared to an average yield of 4.14 x 10^3 for HSV1716 (equivalent to the inoculation dose). The highest 72h yield of HSV1716 obtained in the primary tumour cultures was 1.4x10^5 pfu/10^6 cells in case 2 and the lowest was 7.9x10^4 pfu/10^6 cells in case 1.

13) Table 1 (attached) column 7 shows the 72h yield of 17$^+$ from 10^6 BHK cells over the virus yield from the same number of tumour cells. The tumour cell line MCF-7 supported a wild type HSV infection better than BHK cells, and most of the cultures (primary and established) were fully permissive. Column 8 shows the 72h yield of HSV1716 from BHK cells over the yield from the tumour cells. The yield is the amount of virus released by 10^6 cells, 72h after infection at a multiplicity of infection of 0.1 pfu/cell. For example, in case 1 the yield of 17$^+$ was 1x10^2 lower than in BHK cells and the yield of 1716 was 1.3x10^4 lower than in BHK cells. Therefore, the case 1 culture is impaired in its replication of HSV *per se*, but additionally it is selectively less permissive for ICP34.5-null HSV replication. Also shown (where available) are PCNA PIs *in vitro*. NA= not applicable; ND= not determined.

14) It can be seen that the metastatic tumour samples (cases 1-10) were generally permissive for HSV1716 replication. In three of the cases (1, 5 and 6)

the yield of HSV1716 was more than 1,000-fold lower than in BHK cells. In case 5, this is due to failure of HSV replication *per se* and only in cases 1 and 6 is there a selective disadvantage for HSV1716 replication. Experimental error may account for the cases where there is poor replication of both HSV17$^+$ and HSV1716. The cells were counted prior to plating, therefore poor plating efficiency could lead to cell numbers being lower than calculated. HSV1716 replicated in all of the glioblastoma cultures (cases 11-14), although 11 and 12 were only semi-permissive. These results demonstrate lytic replication of HSV1716 in human metastatic cerebral tumours. In case 9, cells taken from patients' G and P were later shown to be non-neoplastic and are therefore not included in Table 1.

15) Of the cancer cell lines examined, the MCF-7 breast cancer line was fully permissive for HSV1716 whilst the ovarian (SCOV-3), prostate (LNCaP), colon (HT29) and melanoma (C8161) lines were less permissive than BHK cells. The SCOV-3 cell line was semi-permissive for HSV *per se*, yielding two orders of magnitude less than the metastatic ovarian tumour (Case 8), which was fully permissive for both wild type and mutant virus.

16) While not wishing to be bound to any explanation of the mechanism of action, the ability of ICP34.5-null HSV to replicate is thought to depend on the host cell containing PCNA in the active form present in dividing cells (Brown *et al.*, J Gen. Virol.1997 Dec; 71(12): 9442-9449). In addition, in some cells, ICP34.5 appears to be required to preclude the shutoff of cellular protein synthesis (Chou *et al.*,

Proc. Natl. Acad. Sci. USA 1995 Nov 7; 92(23):10516-20). In this case, infection with ICP34.5-null HSV is believed to cause the shutoff of protein synthesis, killing the cells. The two mechanisms likely provide a double hit phenomenon where cells not killed by lytic replication may be killed by the host cell defenses shutting down protein synthesis.

17) This work demonstrates that, in general, human metastatic brain tumours support HSV 1716 infection *in vitro* and that the mode of cell death is by virus replication and cell lysis.

18) From this work, I believe that there is no a *priori* reason why cerebral metastatic tumours of diverse origins should not be treatable by HSV1716 and indeed that they may be more susceptible to oncolysis than glioblastomas.

19) I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By S. Moira Brown

8th May '02 S. Moira Brown, BSc, Ph.D, FRCPath, FRSE

Date:

Table 1: Summary of results.

Case No. /cell line	Age & Sex	Diagnosis	Tumour origin	Tumour site	PCNA PI (%)	72h yield of 17^{+} in BHK/ tumour biopsy or cell line	72h yield of 1716 in BHK/ tumour biopsy or cell line	PCNA PI (%) in culture
1	66 F	Metastatic melanoma	Skin	Right frontal	37.8	$1.0x10^{2}$	$1.3x10^{4}$	ND
2	22 M	Metastatic melanoma	Skin	Right parietal	24.9	4.2	4.6	1.0
3	20 F	Metastatic melanoma	Skin	Right frontal	53.6	4.9	6.5	2.9
4	55 M	Metastatic renal cell carcinoma	Kidney	Right frontal	6.4	2.6	2.0	ND
5	71 M	Metastatic carcinoma	?Lung	Posterior fossa	37.7	$5.4x10^{2}$	$1.5x10^{3}$	ND
6	62 M	Metastatic carcinoma	unknown	Right parietal	19.2	7.4	$1.1x10^{2}$	ND
7	70 M	Metastatic carcinoma	?Lung	Posterior fossa	26.3	5.5	$8.3x10^{1}$	ND
8	51 F	Metastatic adenocarcinoma	Ovary	Occipital lobe	17.5	4.4	6.6	0.3
9	55 F	Metastatic adenocarcinoma	Large bowel	Left parietal	34.9	8.7	$1.0x10^{3}$	0.1
10	61 M	Metastatic	?Lung	Occipital lobe	28.4	7.6	$6.0x10^{3}$	ND

Case No. /cell line	Age & Sex	Diagnosis	Tumour origin	Tumour site	PCNA PI (%)	72h yield of 17^e in BHK/ tumour biopsy or cell line	72h yield of 1716 in BHK/ tumour biopsy or cell line	PCNA PI (%) in culture
		adenocarcinoma						
11	72 M	Glioblastoma Multiforme	Intrinsic	Left frontal	7.7	$1.5x10^1$	$1.6x10^3$	ND
12	70 F	Glioblastoma multiforme	intrinsic	Frontal lobe	13.2	$2.2x10^1$	$5.0x10^3$	ND
13	63 M	Glioblastoma multiforme	intrinsic	Right parietal	17.0	4.0	3.7	3.3
14	47 M	Glioblastoma multiforme	Intrinsic	Right temporal	N/A	5.0	$2.0x10^1$	ND
MCF-7	Adult F	Adenocarcinoma	Breast	Mammary gland	N/A	$9.0x10^{-1}$	4.8	23.3
SCOV-3	Adult F	Adenocarcinoma	Ovary	Ovary ascites	N/A	$2.3x10^2$	$3.2x10^3$	21.1
LNCaP	Adult M	Carcinoma	Prostate	L supraclavicular lymph node	N/A	2.2	$2.2x10^2$	38.8
HT29	44 F	Adenocarcinoma	Colon	Colon	N/A	1.0	$6.5x10^1$	ND
C8161	N/A	Melanoma	Skin	N/A	N/A	7.7	$1.3x10^2$	ND
3T6	mouse	N/A	Embryo fibroblast	N/A	N/A	7.8	$1.8x10^5$	ND

[redacted] Lab.No.:

Surname: F
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower B (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: RIGHT FRONTAL LESION

Macro:

A: Nodule of reddish brown tissue 2 x 1.5 x 1.3cm with cystic cut surface.
B: Similar tissue to specimen A, similar dimensions.

Micro:

A&B: Extensively haemorrhagic and necrotic malignant tumour composed of sheets of large polygonal cells with round to oval nucleus containing a single large nucleolus and vaguely basophilic cytoplasm. There are scattered mitoses. Immunostains for S-100 protein and for the melanoma markers HMB-45 and Melan-A are positive. Stains for cytokeratin EMA and GFAP are negative. The appearance is that of metastatic malignant melanoma.

Conclusion: Metastatic malignant melanoma.

TX2202 M8720/6

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CASE 1

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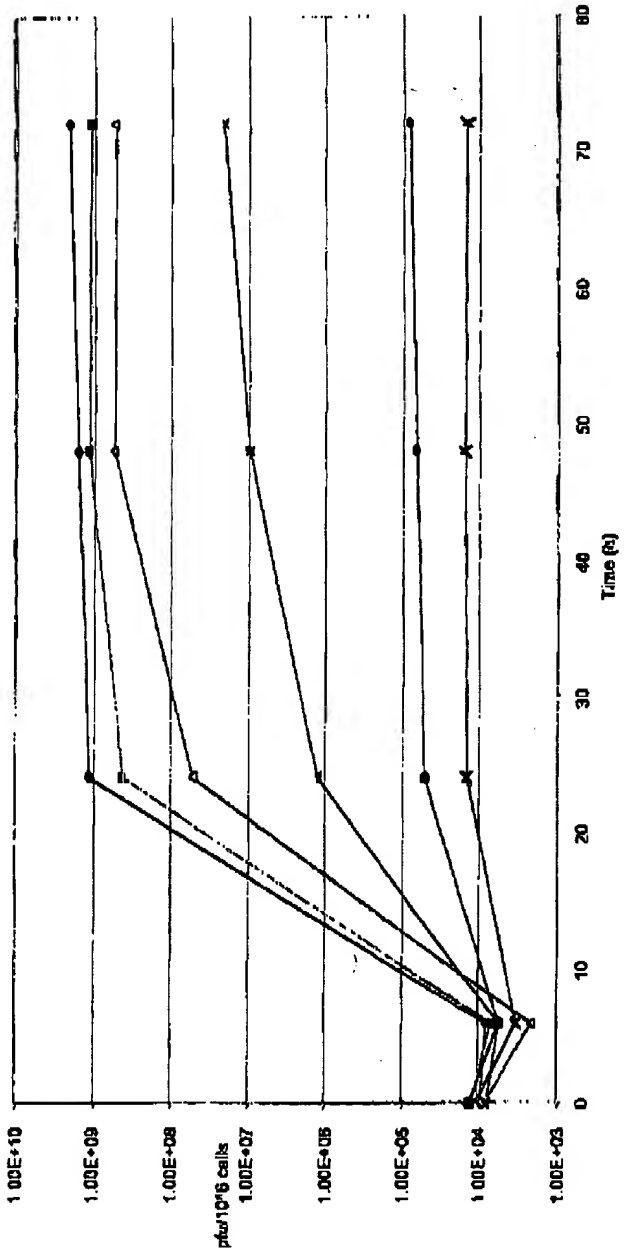
pcb10/97

CASE 1

Virus growth



[redacted] Lab.No.:

Surname: S
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: NCCU (Neuro Critical Care)
Department: Neurosurgery
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: RIGHT PARIETAL LESION

Macro:

A. Tumour - Irregular pieces of haemorrhagic material, together about 2cm across.
B. Blood clot - Piece of blood clot 2 x 2 x 0.7cm.

Micro:

A. Sections show partly necrotic and haemorrhagic malignant tumour composed of diffuse sheets of large polygonal cells with round to oval, sometimes irregular, nucleus, granular chromatin, single nucleolus and moderate amounts of cytoplasm. Scattered mitoses are seen. There are no distinguishing architectural features. Immunostains for epithelial, germ cell and lymphoma markers are negative, but S-100 protein and the melanoma markers HMB-45 and Melan-A are positive. The appearance is that of metastatic malignant melanoma.

B. Blood clot only.

Conclusion: Malignant melanoma.

TX2302 M8720/6

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CASE 2

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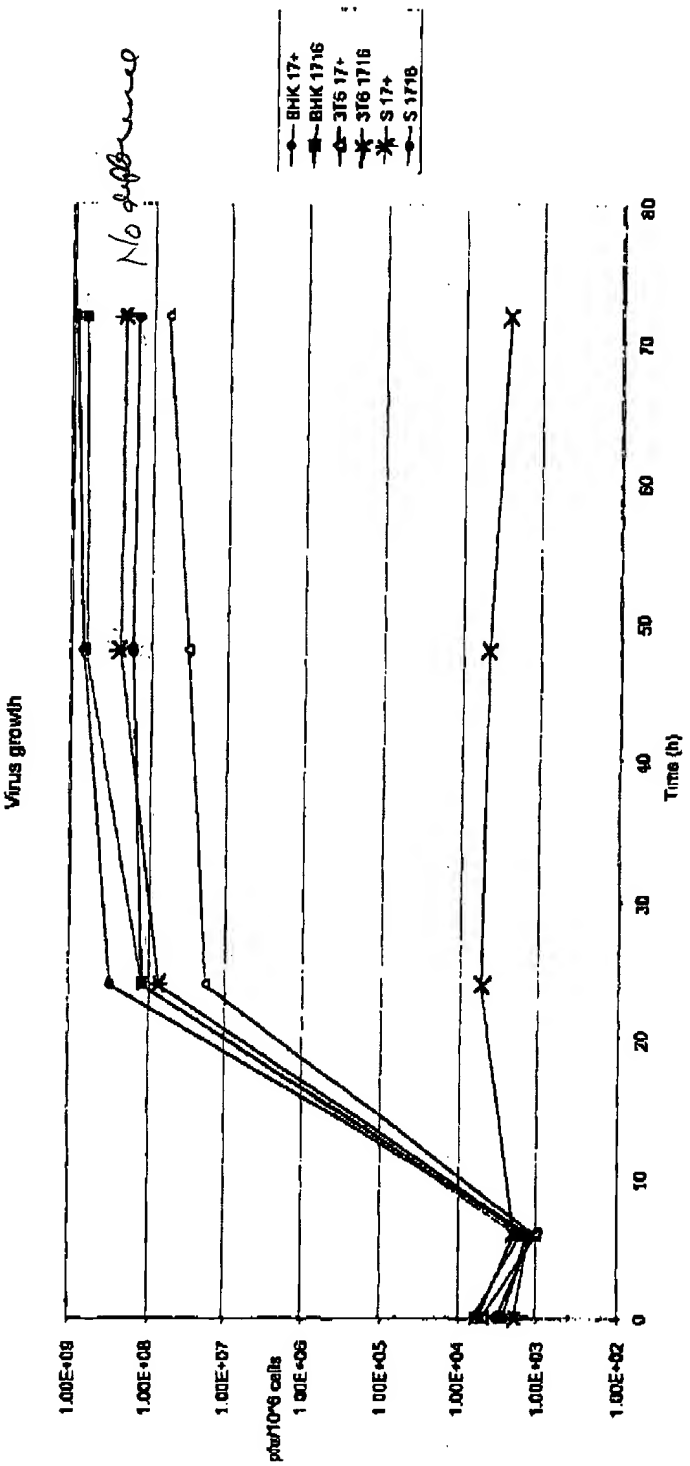
CASE 2


Virus growth
No antibody
BHK 17+
BHK 1716
3T6 17+
3T6 1716
S 17+
S 1716
pfu/10^6 cells
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
0
10
20
30
40
50
60
70
80
Time (h)

Surname: R
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower B (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: RIGHT FRONTAL LESION

Macro:

Red nodule with white foci 2.5 x 2 x 1.5cm.

Micro:

Section shows a melanotic melanoma with haemorrhage and necrosis, consistent with a metastasis.

TX2202 M8720/6

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CASE 3

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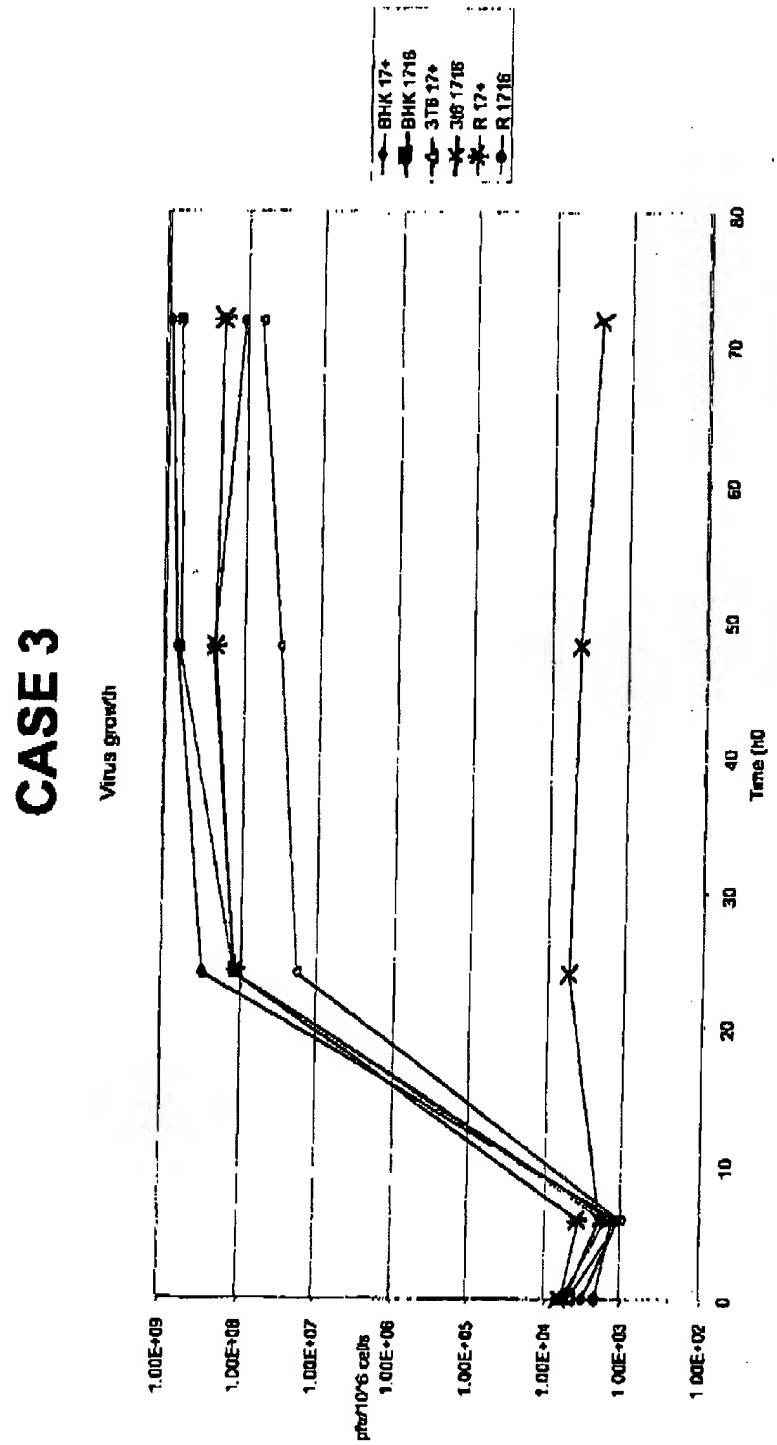

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Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower A (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

Nature of Specimen: RIGHT FRONTAL LESION

Macro:

A. Haemorrhagic tissue 3 x 2 x 2cm. There are yellow areas on cut surface.
B. Similar tissue 4 x 4 x 2cm.

Micro:

A and B show metastatic carcinoma that is mainly clear cell, with papillary foci. It is consistent with origin from a renal primary tumour.

Diagnosis: Metastatic renal cell carcinoma.

TX2202 M8310/6

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NEUROPATHOLOGY

CASE 4

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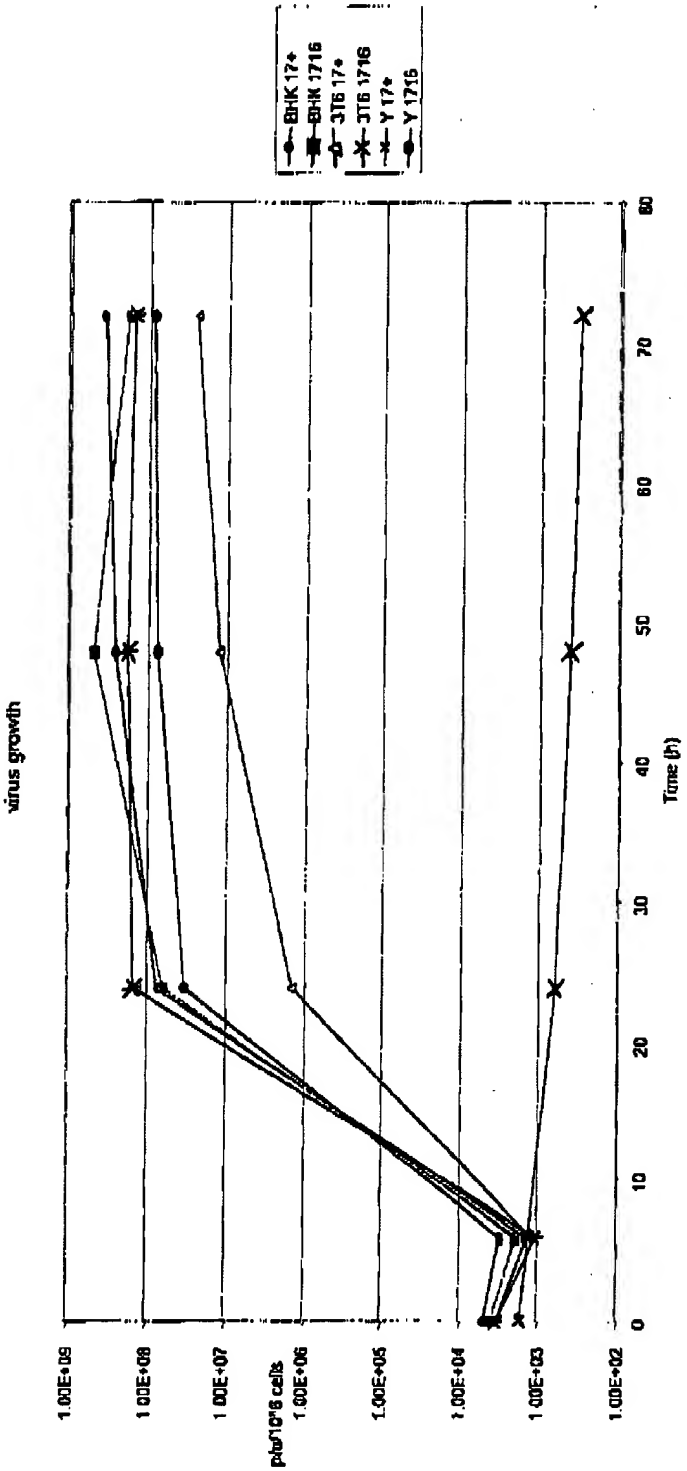
CASE 4



[redacted] Lab.No.:

Surname: B
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower B (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: POSTERIOR FOSSA LESION

Macro:

Fragments of soft, friable tissue together about 2cm across.

Micro:

Sections show partly necrotic, poorly differentiated metastatic carcinoma composed of sheets of large polygonal cells with no obvious architectural pattern. In places the tumour cell nuclei are very large and bizarrely shaped and there are multinucleate tumour giant cells. Site of origin cannot be determined, but lung would be a likely possibility.

Conclusion:

Metastatic carcinoma.

TX6000 M8010/6

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CASE 5

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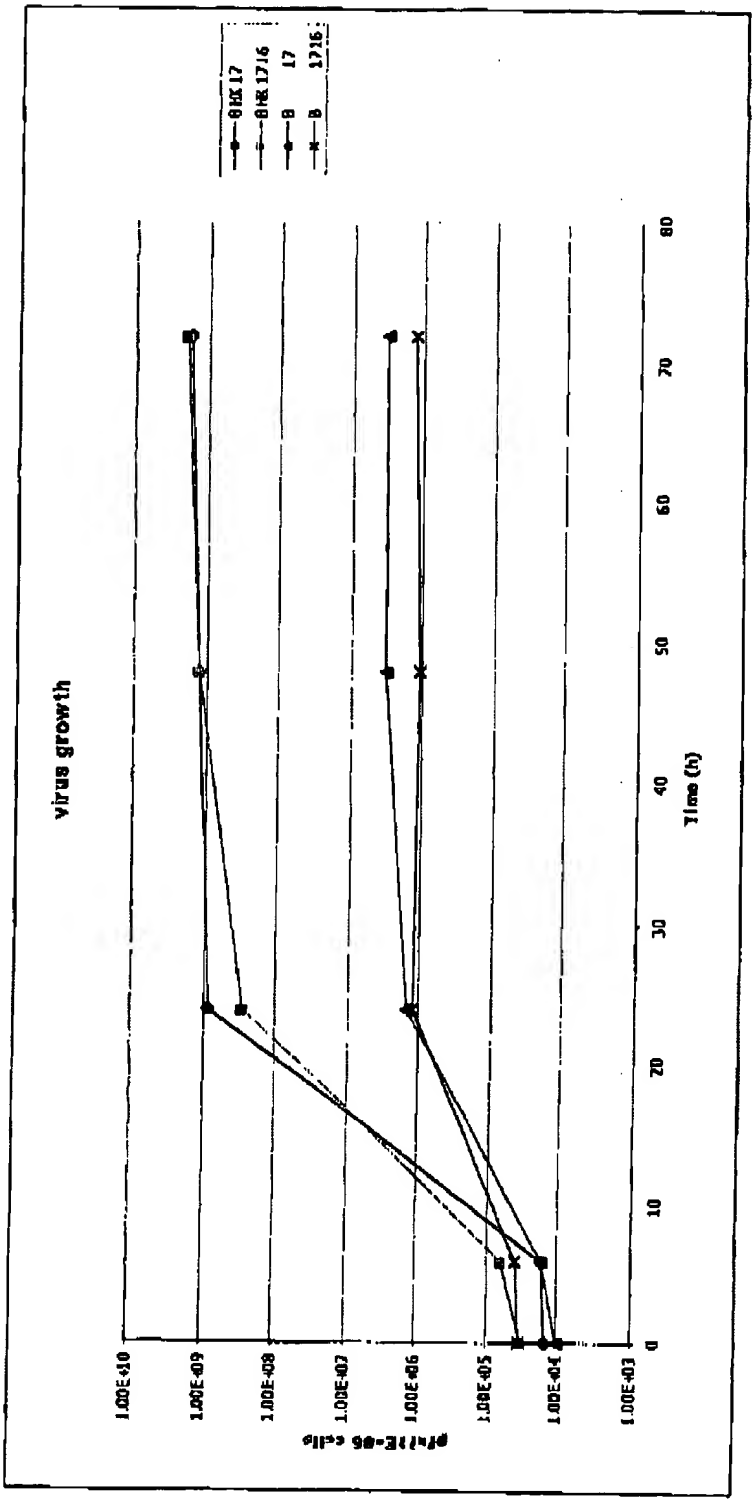
CASE 5



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Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower B (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: RIGHT PARIETAL LESION

Macro:

Irregular piece of firm grey tissue 1.5 x 0.9 x 0.8cm maximum dimension and a few tiny fragments.

Micro:

Sections show partly necrotic metastatic carcinoma set in heavily gliotic brain tissue. The appearance is more suggestive of squamous carcinoma than adenocarcinoma, but it is difficult to be certain.

Conclusion: Metastatic carcinoma.

TX2302 M8010/3

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CASE 6

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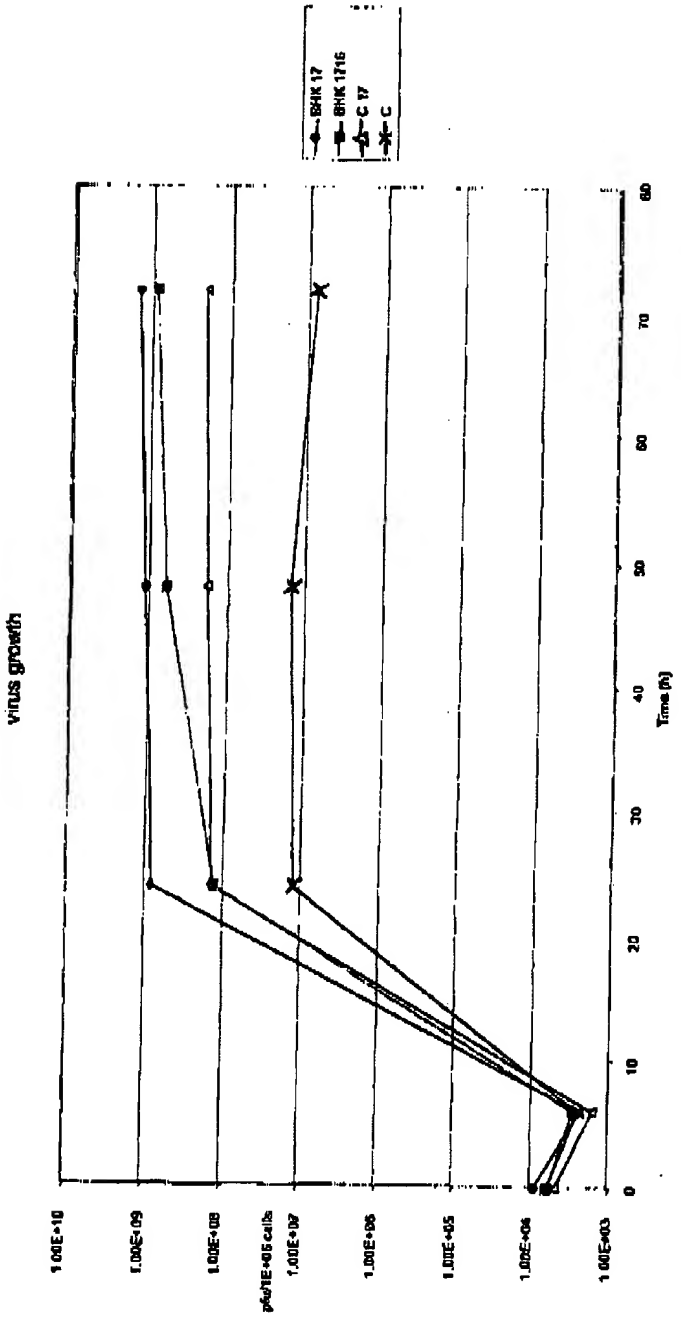
CASE 6



[redacted] Lab.No.:

Surname: K
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower A (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

Nature of Specimen: POSTERIOR FOSSA LESION

Macro:

Irregular piece of firm grey tissue 2.5 x 1.5 x 1cm, with 2 separate small fragments.

Micro:

Sections show extensively necrotic metastatic poorly differentiated carcinoma, entirely consistent with lung primary origin. Other origins cannot be excluded.

Comment: I am unsure of the exact histological type of carcinoma here. Squamous seems more likely than adenocarcinoma. In any event this is not small cell carcinoma.

Conclusion: Metastatic carcinoma.

TX6000 M8140/6

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NEUROPATHOLOGY

CASE 7

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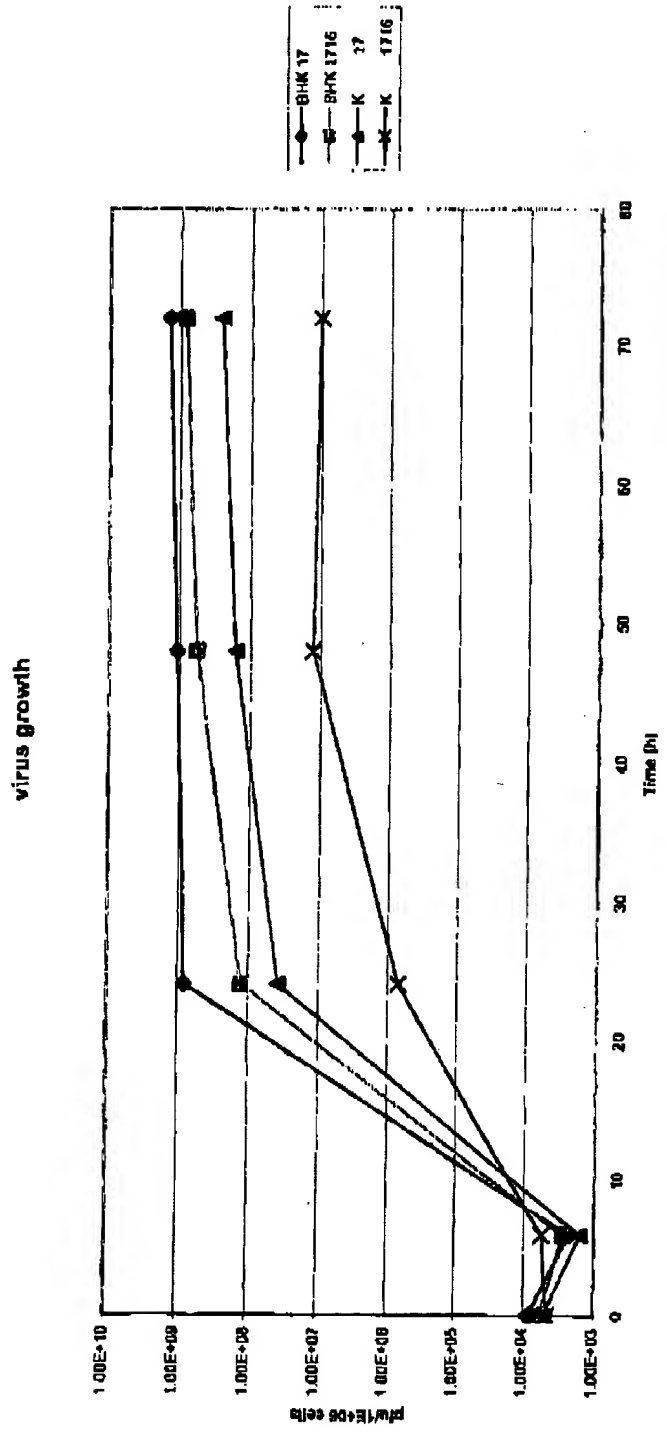
CASE 7



[redacted] Lab.No.:

Surname: N
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower B (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: OCCIPITAL LESION

Macro:

Nodular mass 2 x 1.3 x 1cm.

Micro:

Section shows partly necrotic adenocarcinoma with many mucinous cells and in places there is a papillary pattern.

It is consistent with a metastasis from primary ovarian tumour.

TX2403 M8140/6

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CASE 8

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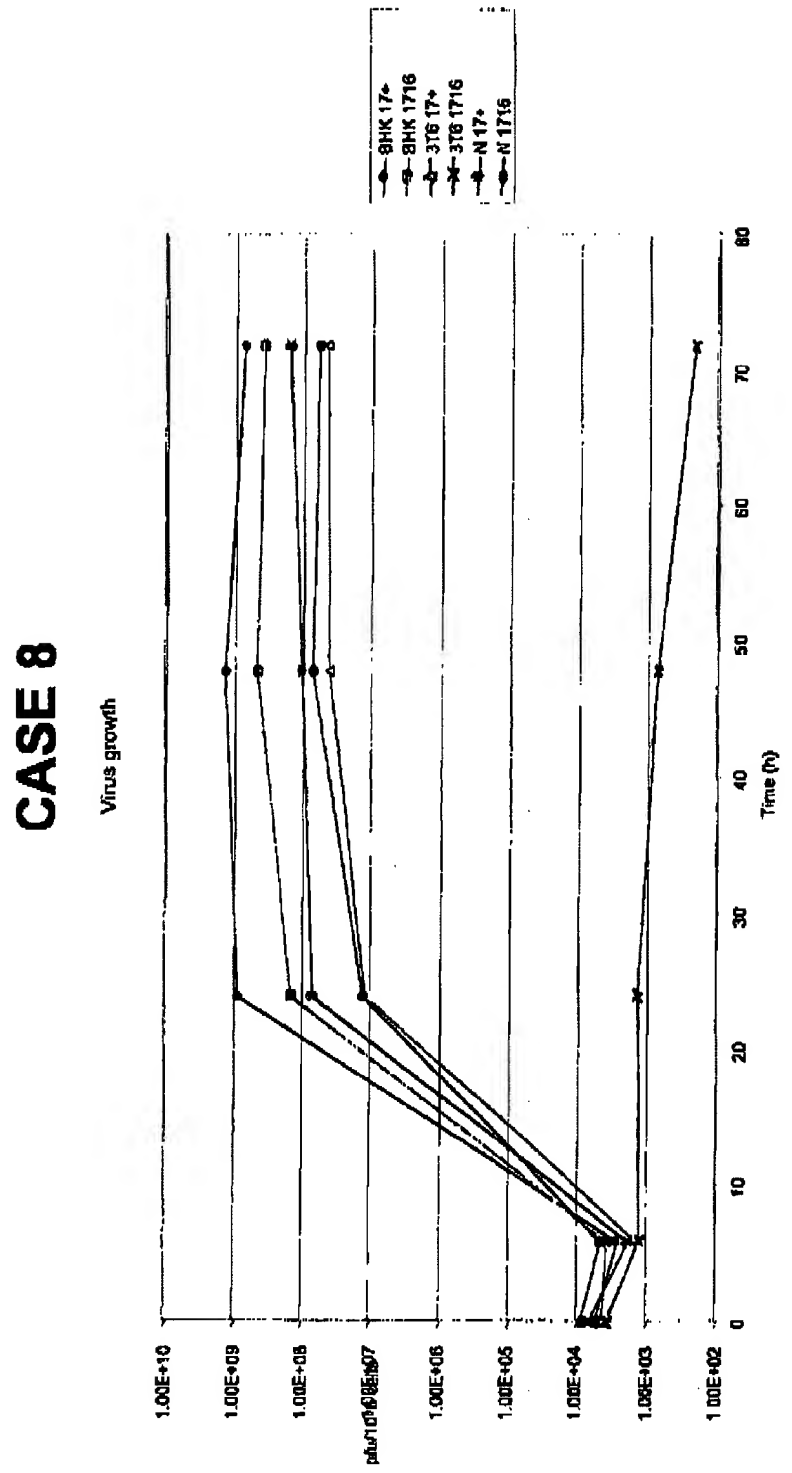
CASE 8
Virus growth
3HK 17+
3HK 1716
3T6 17+
3T6 1716
N 17+
N 1716
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
0
10
20
30
40
50
60
70
80
Time (h)

[redacted] Lab.No.

Surname: K1
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: Queen Elizabeth Hospital, B'ham
Ward: Ward East Lower E (Neurosurg)
Department: Neurosurgery
Ext. Reference:
Date Received:

Nature of Specimen: LEFT PARIETAL LESION

Macro:

Irregular piece of soft, grey tissue 2 x 1.5 x 0.9cm maximum dimensions.

Micro:

Ghotic brain tissue containing areas of extensively necrotic metastatic adenocarcinoma, whose appearance is consistent with large bowel origin.

+

TX2303 M8140/6

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NEUROPATHOLOGY

CASE 9

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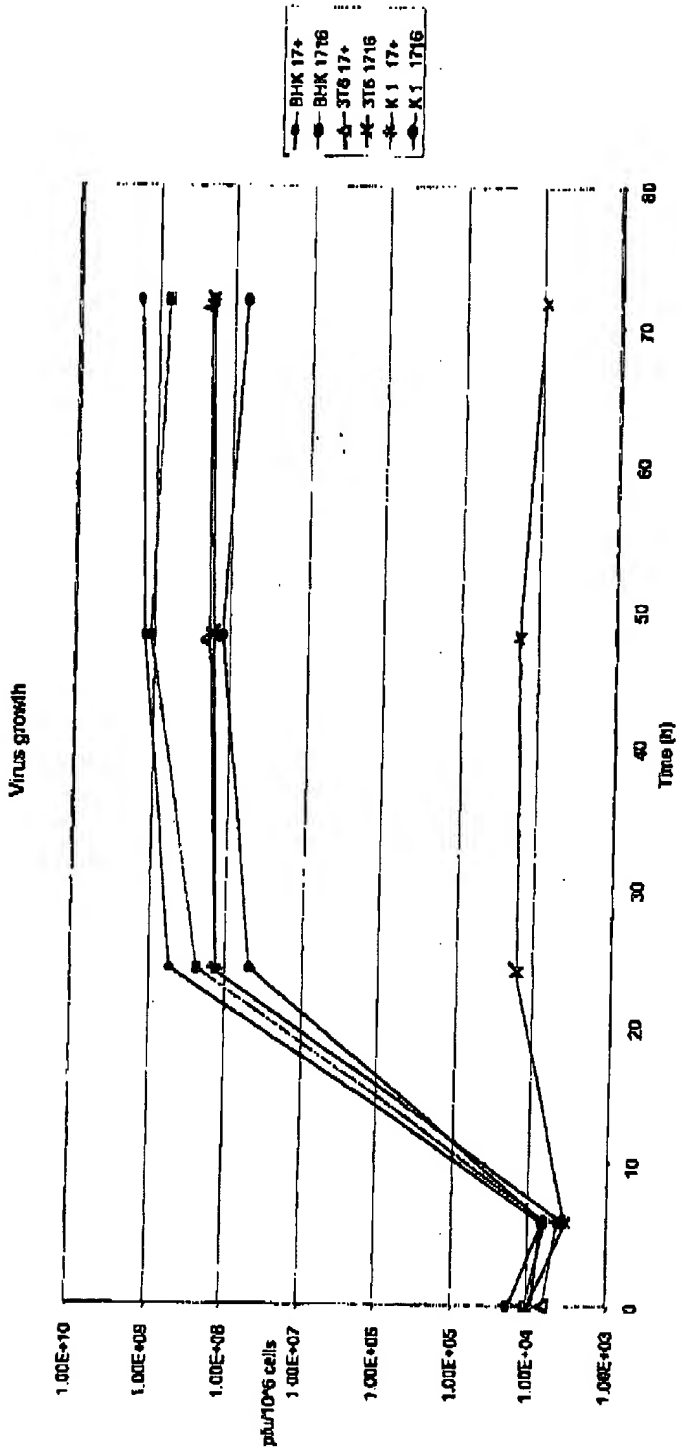
CASE 9



[redacted] Lab.No.

Surname: P1
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **NCCU (Neuro Critical Care)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

Nature of Specimen: SPENOIDAL LESION

Macro:

A - Irregular yellow tissue 0.6cm.
B - Pieces of irregular yellow and brown tissue 2cm.

Micro:

A. Section shows fragment of actively inflamed granulation tissue.

B. Section shows densely gliotic brain attached to actively inflamed collagen and granulation tissue. No organisms are seen on special stains but the appearances indicate infection. No definite evidence of neoplasia is seen.

TX2500 M4300

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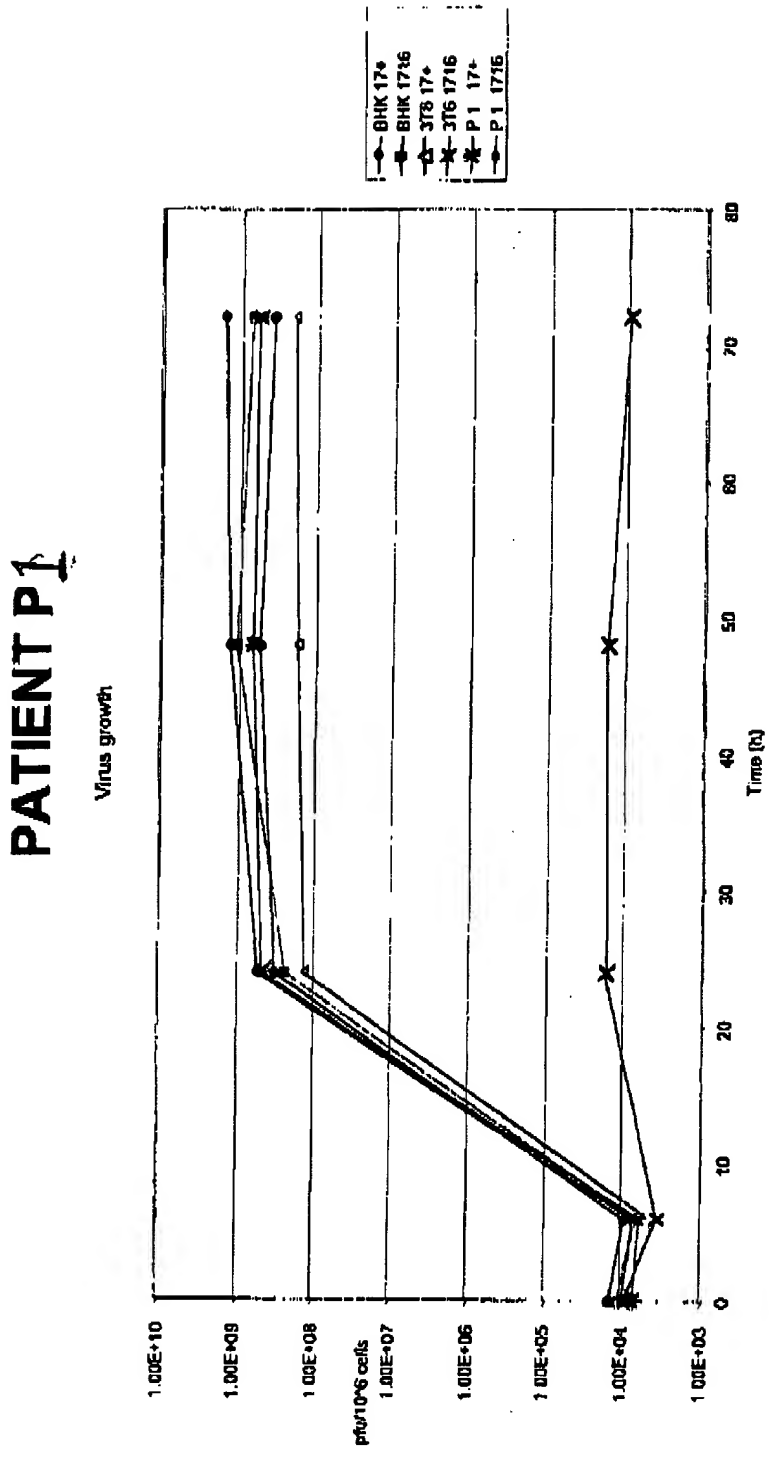

[redacted] Lab.No.:

Surname: G
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower B (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: RIGHT OCCIPITAL LESION

Macro:

Nodule of firm, pale tissue, 1.5cm in diameter, slightly ragged external surface. Cut surfaces show patchy areas of necrosis.

Micro:

Sections show a mass of confluent necrotizing granulomatous inflammation with a thin rim of gliotic brain tissue in places. The granulomas contain masses of epithelioid cells and lymphocytes with well developed Langhans giant cells and large irregular areas of necrosis. Stains for bacterial and fungal organisms, including Ziehl-Neelsen stain for acid fast bacilli, are negative.

Comment:

In spite of the negative staining, this is almost certainly an infective process with tuberculosis by far the most likely organism. Other organisms such as yeasts and other fungi, spirochaetal infections etc cannot be excluded but are much less likely.

Conclusion:

Necrotising granulomatous inflammatory process, most likely tuberculosis. Other causes cannot be excluded.

TX2402 M44000

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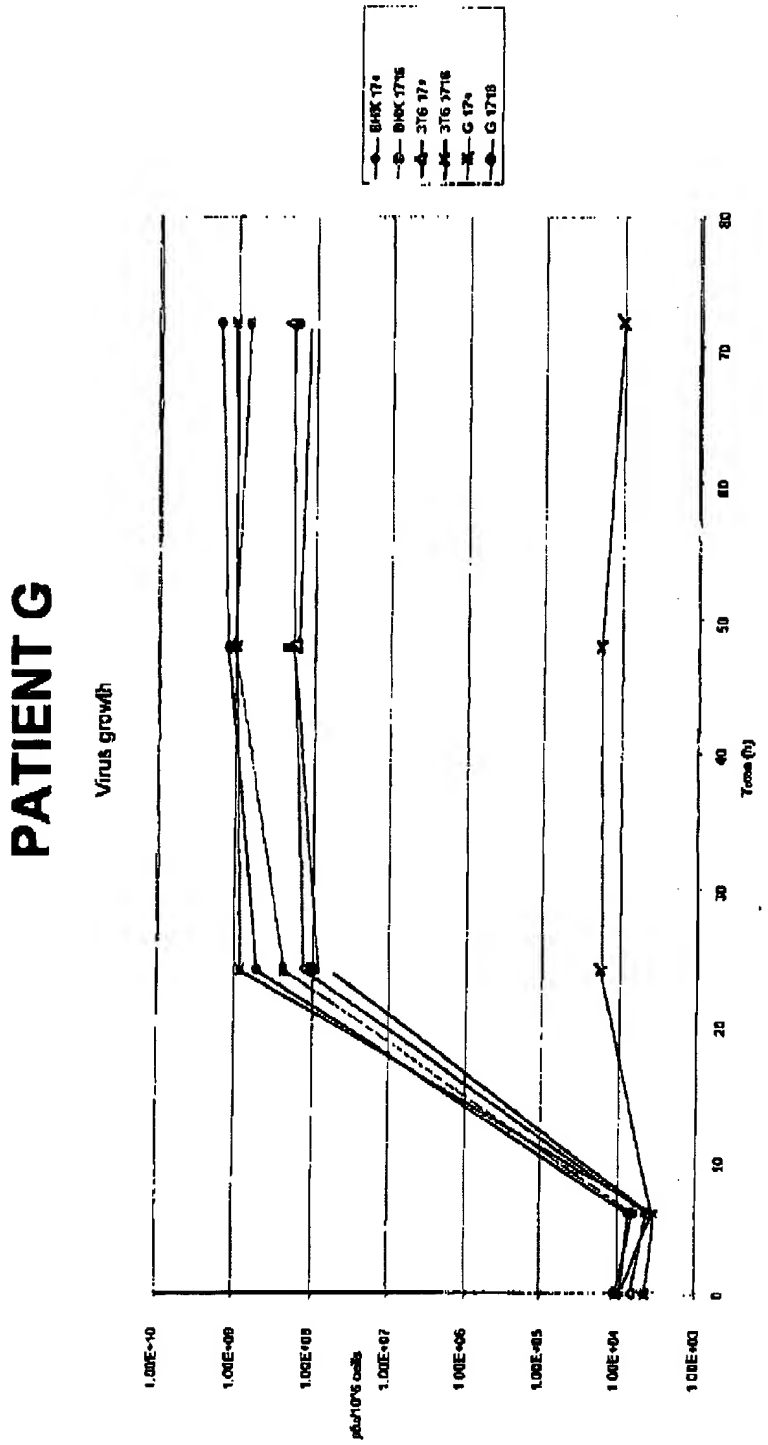

[redacted] Lab.No.:

Surname: E
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant: J
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower B (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: OCCIPITAL LOBE TUMOUR

Macro:

Fragments of pale and brown soft tissue together about 1.8cm.

Micro:

Sections show poorly differentiated metastatic adenocarcinoma. Lung would be one possible primary origin, but other origins should also be considered.

TX2400 M8140/6

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CASE 10

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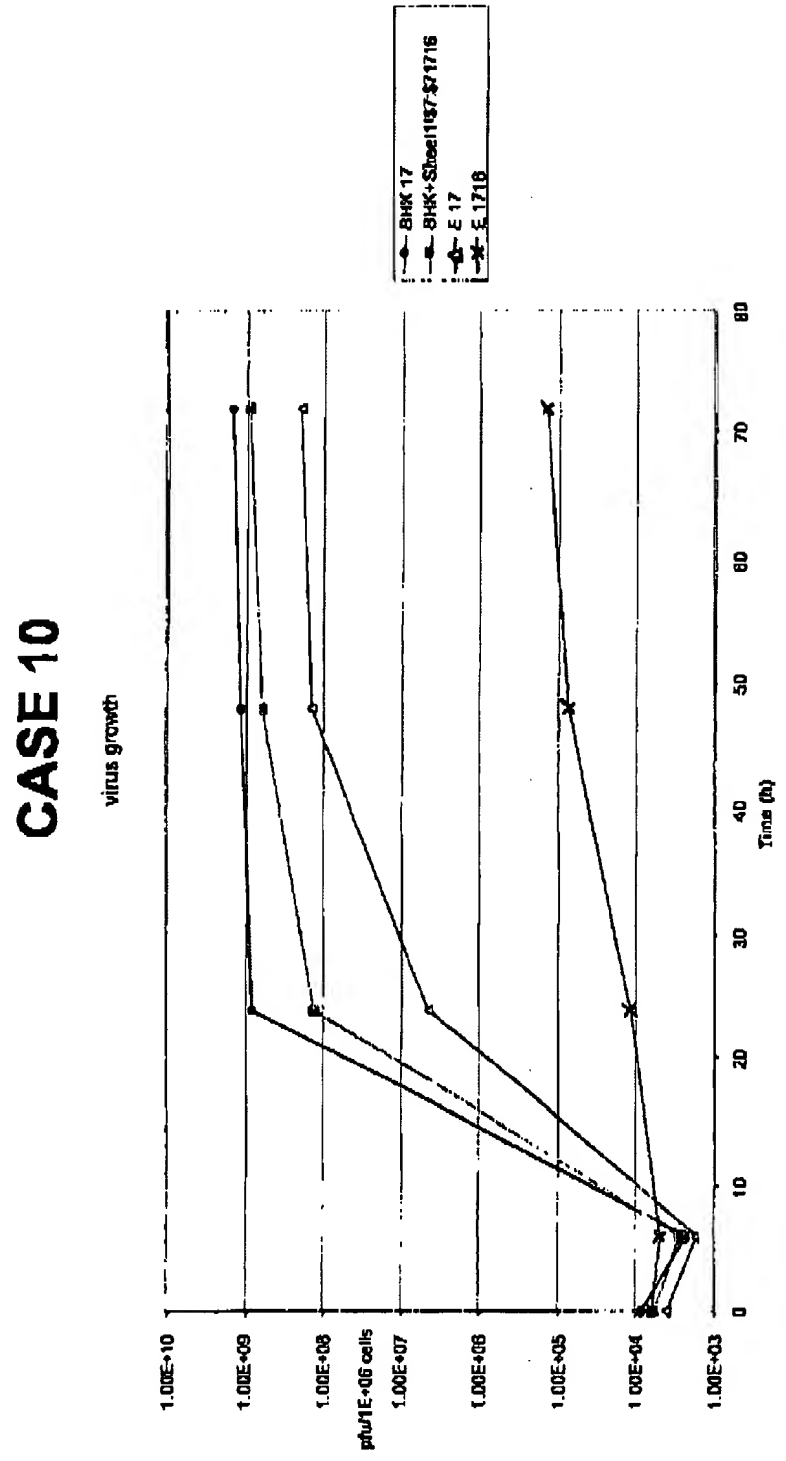
CASE 10



[redacted] Lab.No.:

Surname: S1
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **NCCU (Neuro Critical Care)**
Department: **Neurosurgery**
Ref. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: LEFT FRONTAL LESION

Macro:

A) "Residual tumour ?" - irregular grey white tissue 1.3cm across.
B) "Normal tissue tumour" - grey and white tissue 1.6cm across.
C) Irregular cerebral tissue 2cm across.

Macro:

A, B and C show cerebral tissue bearing a fairly cellular astrocytic tumour with small, anaplastic nuclei, mitotic activity, several figures of serpiginous necrosis and florid microvascular (vascular endothelial) hyperplasia.

As it was removed in several pieces it is difficult to comment on completeness of excision.

Diagnosis:

Glioblastoma (astrocytoma grade 4).

TX2203 M940/3

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CASE 11

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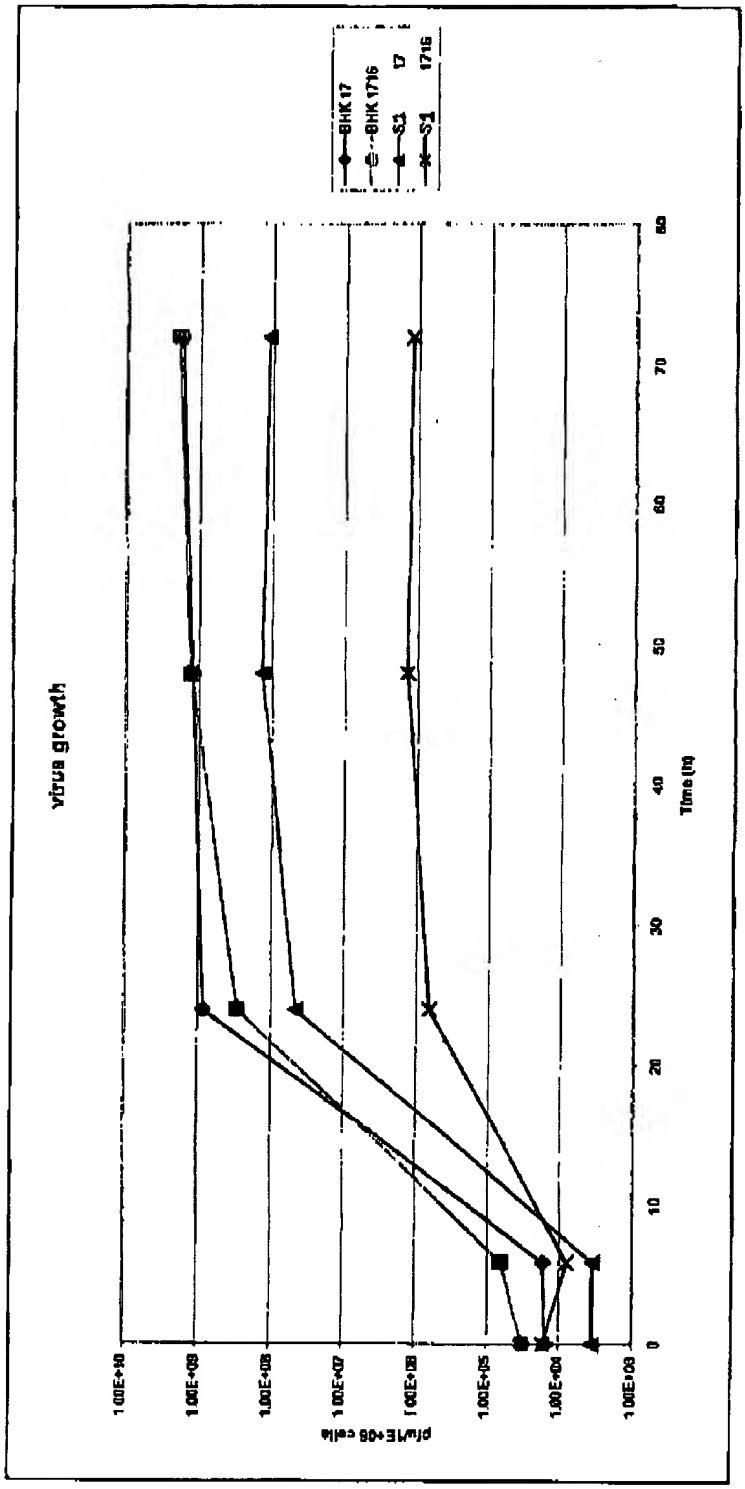
CASE 11



[redacted] Lab.No.:

Surname: P
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower A (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

NEUROPATHOLOGY

Nature of Specimen: FRONTAL LOBE LESION

Macro:

A: Fragments of soft grey tissue together 1.2 x 1 x 0.5cm.
B: Frontal lobectomy specimen 7 x 5 x 3.5 maximum dimension. Cut surfaces show normal looking grey and white matter.

Micro:

A: Sections show malignant glioma of moderate to high cellular density composed of cells with markedly pleomorphic hyperchromatic nuclei and fibrillary cytoplasm. There are mitoses and apoptotic bodies, capillary endothelial proliferation and areas of necrosis. The appearance is that of glioblastoma. Stains for organisms are negative.

B: Cerebral cortex and subjacent white matter showing patchy infiltration by glioblastoma in several areas along the deep margin of the specimen.

Conclusion: Glioblastoma multiforme (astrocytoma grade 4).

TX2200 M9440/3

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CASE 12

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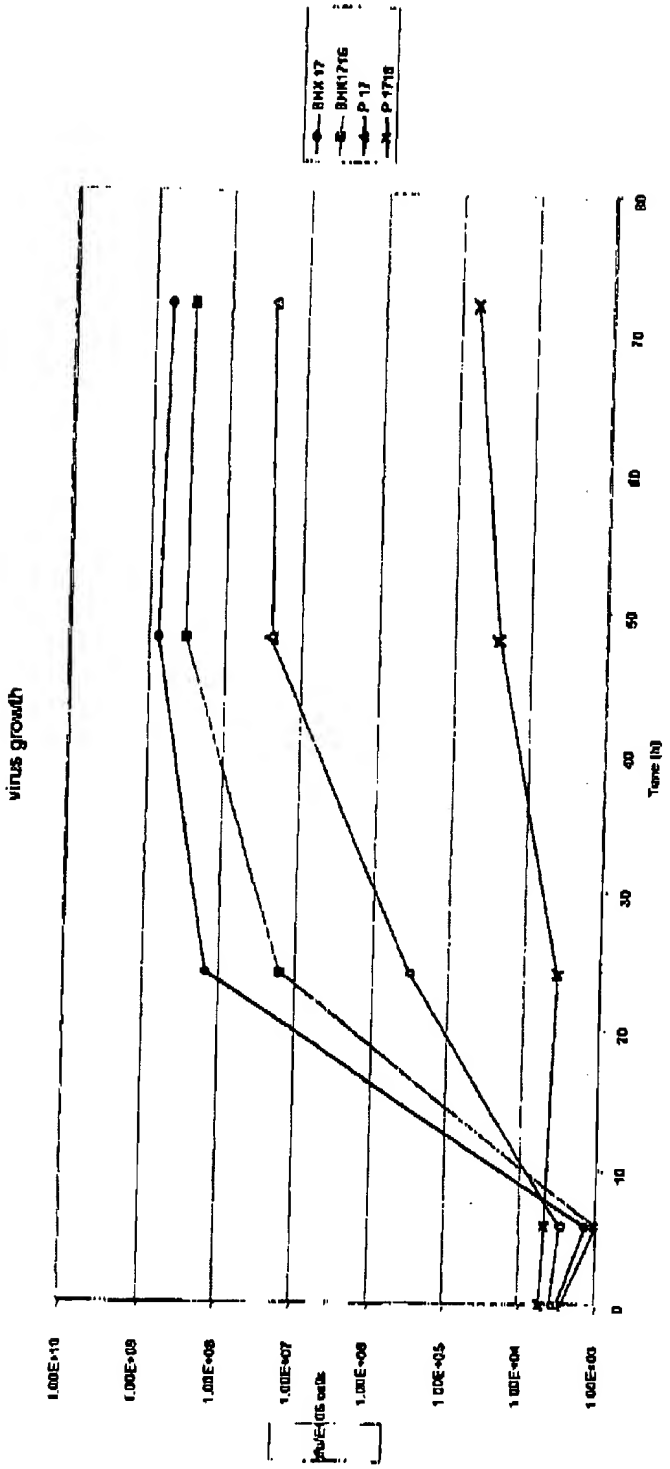
CASE 12



[redacted] Lab.No.:

Surname: M
Forename:
Date of Birth:
Sex:
Reg. Number:
NHS Number:

Consultant:
Hospital: **Queen Elizabeth Hospital, B'ham**
Ward: **Ward East Lower B (Neurosurg)**
Department: **Neurosurgery**
Ext. Reference:
Date Received:

Nature of Specimen: RIGHT PARIETAL LESION

Macro:

Pieces of soft, grey tissue, some are small and two are up to 1cm.

Micro:

Section shows a cellular tumour composed of small, anaplastic glial cells with mitotic activity. There is geographical and serpiginous necrosis, and abundant microvascular hyperplasia is present.

There is also a tangle of large, atypical vessels reminiscent of an A-VM.

Diagnosis: Glioblastoma (astrocytoma grade 4).

TX2302 M9440/3

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NEUROPATHOLOGY

CASE 13

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CASE 13